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STATE OF ISRAEL

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רצופים בזה העתקים
נכונים של המסמכים
שהופקדו לכתחילה
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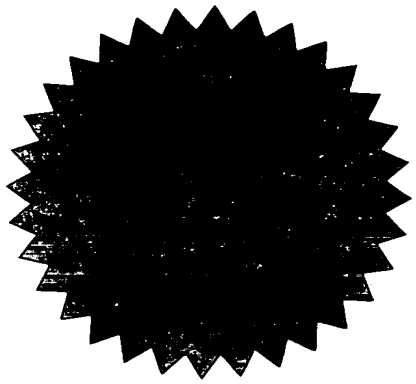

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נתאשר

חוק הפטנטים, תשכ"ז - 1967
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בקשה לפטנט
Application For Patent

לשימוש הלשכה
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אני, (שם המבקש, מענו ולגבי גוף מאוגד - מקום התאגדותו)
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בעל אמצאה מכח הדין a right of law ששמה הוא
Owner, by virtue of of an invention the title of which is

(בעברית) (Hebrew)
נוגדנים כנגד HBV

(באנגלית) (English)
Anti HBV antibodies

מבקש בזאת כי ינתן לי עליה פטנט hereby apply for a patent to be granted to me in respect thereof.

* בקשת חלוקה – Application of Division	* בקשת פטנט מוסף – Appl. for Patent of Addition	* דרישת דין קדימה Priority Claim		
מבקשת פטנט from application	* לבקשה/לפטנט to Patent/Appl.	מספר/סימן Number/Mark	תאריך Date	מדינת האיגוד Convention Country
No. מס'	No. מס'			
dated מיום	dated מיום			

P.O.A. : * יפוי כח : עוד יוגש

filed on a previous case הוגש בענין קודם

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היום 11th בחודש June שנת 1996
This of the year

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* מחק את המיותר

נוגדנים כנגד HBV

Anti HBV antibodies

אקס טי אל ביופארמאסוטיקלס בע"מ

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ANTI HBV ANTIBODIES

FIELD OF THE INVENTION

The present invention concerns a process for obtaining hybridoma cell lines which produce human antibodies capable of binding to the hepatitis B virus surface antigen, the hybridoma cell lines, antibodies produced by the cell lines, and various uses thereof.

BACKGROUND OF THE INVENTION

Hepatitis B virus (HBV) infection is a major worldwide health problem. Approximately 5% of the world population is infected by HBV and chronically infected patients carry a high risk of developing cirrhosis and hepatocellular carcinoma. (Progress in Hepatitis Research: Hepatitis B virus (HBV), Hepatitis C virus (HCV) and Hepatitis Delta virus (HDV) Ed. O. Crivelli, Sorin Biomedica, 1991).

The immune response to HBV-encoded antigens includes both a cellular immune response which is active in the elimination of HBV infected cells, as well as a humoral antibody response to viral envelope antigens which contributes to the clearance of circulating virus particles. The dominant cause of viral persistence during HBV infection is the development of a weak antiviral immune response.

Recombinant HBV vaccines provide a safe and effective means for active immunization against HBV, however, they do not always induce a sufficient and rapid antibody response.

Interferon-α has been used in the therapy of Hepatitis B infection showing an efficacy of only 30-40% in highly selected patients.

In addition, passive immunization with human polyclonal anti Hepatitis B antisera has been shown to be effective in delaying and even preventing recurrent HBV infection (Wright, T.L. and Lau, J.Y.N. The Lancet 342:1340-1344, (1993)). Such human polyclonal antisera are prepared from pooled plasma of immunized donors. These preparations are very expensive and available in relatively small amounts. Furthermore, pooled plasma may contain contaminated blood samples and thus treatment with such antisera increases the patient's risk to contract other viral infections such as hepatitis C or HIV.

An alternative approach for the treatment of HBV infection is the use of monoclonal antibodies (MoAb).

PCT patent application PCT/NL94/00102 discloses human monoclonal antibodies directed against Hepatitis B surface antigen which are secreted by the hybridoma cell lines Mab 4-7B and Mab 9H9. The monoclonal antibody secreted by the cell line Mab 4-7B recognizes a linear epitope of HBVsAg and is different from the Mab 9H9 monoclonal antibody which recognizes a conformational epitope. The antibodies are claimed for simultaneous use in the treatment of chronic Hepatitis B infections.

PCT patent application PCT/US92/09749 discloses human monoclonal antibodies against HBVsAg which are secreted by the hybridoma cell lines PE1-1, ZM1-1, ZM1-2, MD3-4 and LO3-3. The antibodies bind to different HBV epitopes and are used for reducing the level of circulating HBVsAg.

Japanese Patent Application JP 93066104 discloses a hybridoma of a human lymphocyte cell strain TAW-925 and a human lymphocyte transformed by Epstein-Barr virus. The hybridoma produces a human monoclonal antibody against HBVsAg.

U.S. Patent Application No. 4,883,752 discloses preparation of human-derived monoclonal antibody to HBVsAg, by administration of HBVsAg vaccine to humans, recovering their lymphocytes, stimulating the lymphocytes *in vitro* by a non specific stimulator, fusing said cells with a myeloma cell, and selecting for hybridomas with secrete anti HBVsAg antibodies.

Ichimori *et al., Biochem. and Biophysic. Research Communications* 129(1):26–33, 1985 discloses a hybridoma secreting human anti HBVsAg monoclonal antibodies which recognize the a–determinant of HBVsAg. Later, Ichimori, *et al., supra* 142(3):805–812, 1987 disclosed another hybridoma which stably secretes human monoclonal antibody against HbsAg.

The abovementioned antibodies were all developed by *in vitro* immortalization of antibody–producing cells from individuals positive for anti–HBV antibodies.

A new approach enabling adaptive transfer of human peripheral blood mononuclear cells (PBMC) into lethally irradiated normal strains of mice radioprotected with severe combined immune deficiency (SCID) bone marrow was recently described (Lubin I., *et al., Blood*, 83:2368, 1994). Secondary humoral responses to various recall antigens as well as a primary humoral response to other antigens were shown to be generated effectively in such human/mouse chimeras (Marcus H., *et al., Blood*, 86:398–406, 1995).

SUMMARY OF THE INVENTION

In accordance with the present invention, it was found that hybridoma cell lines secreting human antibodies capable of binding to the Hepatitis B surface antigen (HBVsAg) may be obtained using the abovementioned human/mouse chimeras. In accordance with the present invention, human peripheral blood lymphocytes (PBL) from human donors positive for anti HBVsAg antibodies are engrafted into normal strains of mice which were lethally irradiated and radioprotected with SCID bone marrow. After immunization of such chimeric mice with HBVsAg, human cells are obtained from the mice spleens and fused *in vitro* with heteromyeloma cells to generate hybridomas secreting human antibodies having a high affinity and specificity to HBVsAg.

The present invention thus provides a process for obtaining human monoclonal antibodies (hMoAb) capable of binding to Hepatitis B virus surface antigen (HBVsAg) comprising:

(a) immunizing a chimeric rodent M4 having xenogeneic hematopoietic cells with Hepatitis B surface antigen (HBVsAg) such that xenogeneic

having any combination of the abovementioned hematopoietic deficiencies. In addition, the hematopoietic deficiency may also be a result of gene deletion or transgenic mice may be used.

The hematopoietic cells derived from the donor mouse M2 are preferably bone marrow cells either untreated or depleted of T cells. Other suitable sources of hematopoietic cells which may also be used include, for example, spleen cells, fetal liver cells or peripheral blood cells.

The xenogeneic hematopoietic cells derived from the human M3 are preferably PBL cells but may also be derived from any suitable source of human hematopoietic cells such as bone marrow cells, cord blood cells, thymus spleen or lymphnode cells, etc.

By a most preferred embodiment, the rodent M1 is a mouse or rat, the mouse M2 is a SCID mouse and the xenogeneic hematopoietic cells derived from the human M3 are PBLs from a human M3 which has already been exposed to the HBVsAg either spontaneously as a result of a prior infection or induced following vaccination. Such humans will have a relatively high titer of anti HBVsAg antibodies as compared to individuals which have never been infected with HBV and, therefore, when PBLs from such donors are used as M3 donor cells in accordance with the present invention, the immunization of the M4 chimeric mouse with HBVsAg will elicit a secondary immune response of the transplanted human PBLs in the M4 chimeric mouse. A most preferred human donor M3 is such which tested negative for the HB virus but shows a high titer of antibodies against HBVsAg. Such PBLs from the human M3 donor may be obtained either by whole blood donation or by leukophoresis.

The HBVsAg used for immunizing the chimeric rodent M4 in accordance with the invention is preferably a Hepatitis B virus vaccine containing the purified major surface antigen of the virus prepared by recombinant DNA technology (Engerix™-B, SIB Biological (Rixensart, Belgium)).

The present invention is also directed to hybridoma cell lines producing human monoclonal antibodies capable of binding to HBVsAg, as well as to human monoclonal antibodies capable of binding to HBVsAg and fragments thereof substantially maintaining the antigen binding characteristics of the whole antibody.

antibody-producing cells are produced in said rodent, wherein said rodent M4 is a rodent M1, the hematopoietic cells of which have been substantially destroyed, said rodent M1 having transplanted therein hematopoietic cells derived from a mouse M2 having a hematopoietic deficiency, and xenogeneic hematopoietic cells derived from human M3;

(b) removing and immortalizing said antibody-producing cells;

(c) selecting and cloning the immortalized antibody producing cells producing the antibodies capable of binding to HBVsAg and;

(d) isolating the antibodies produced by the selected, cloned immortalized antibody producing cells.

In accordance with the invention, spleens of the immunized chimeric rodent M4 are removed between 12 and 20 days after human PBL transplantation, preferably at day 14 after transplantation thereof. Cell suspensions are prepared from the spleens and the antibody producing cells obtained from the immunized chimeric rodent M4 are fused preferably with a human-mouse fusion partner such as a heteromyeloma by techniques well known in the art (e.g. Kohler & Milstein, *Nature*, 256:495-497, 1975). In order to isolate the antibodies produced by the selected hybridoma cell lines in accordance with the invention, the hybridoma cell lines are either cultured *in vitro* in a suitable medium wherein the desired monoclonal antibody is recovered from the supernatant or, alternatively, the hybridoma cell lines may be injected intraperitoneally into mice and the antibodies harvested from the malignant ascitis or serum of these mice. The supernatant of the hybridoma cell lines are first screened for production of human IgG antibodies by any of the methods known in the art such as enzyme linked immunosorbent assay (ELISA) or radioimmuno assay (RIA). Hybridomas testing positive for human IgG are then further screened for production of anti HBVsAg antibodies by their capability to bind to HBVsAg.

The M1 rodent in accordance with the invention is preferably a rodent conventionally used as a laboratory animal, most preferably a rat or a mouse.

The mouse M2 may have any hematopoietic deficiency including genetic hematopoietic deficiencies as well as induced hematopoietic deficiencies. Non limiting examples of hematopoietic deficiencies include SCID, Bg, Nu, Xid or mice

obtaining a body fluid sample from the tested individual which may be a blood sample, a lymph sample or any other body fluid sample and contacting the body fluid sample with a human anti HBVsAG antibody of the invention under conditions enabling the formation of antibody-antigen complexes. The level of such complexes is then determined by methods known in the art, a level significantly higher than that formed in a control sample indicating an HV infection in the tested individual. In the same manner, the specific antigen bound by the antibodies of the invention may also be used for diagnosis. In the same manner, the specific antigen of the invention may also be used for diagnosis of HBV infection in an individual by contacting a body fluid sample with the Ag and determining the presence of Ag-Ab complexes in the sample as described above. In addition, the Ag of the invention may be used for immunizing an individual to elicit a humoral response against HBV.

The present invention further provides a kit for use in the therapy of HBV infections or diagnosis of such infections comprising the antibodies of the invention, the antigen bound by the antibodies of the invention and any further reagents necessary for detecting such antibodies or antigens in a tested sample.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graphic representation showing the amount of total human Ig (mg/ml) and the amount of specific anti HBs antibodies (mU/ml) in the sera of irradiated mice which were radioprotected with SCID bone marrow (chimeric mice).

PBL+Engerix: the chimeric mice were further transplanted with human PBL from donors positive for anti HBs antibodies, and vaccinated with Engerix B in an aluminium hydroxide adjuvant (alum).

PBL+Alum: the chimeric mice were further transplanted with human PBL from donors positive for anti HBs antibodies, and vaccinated with Alum alone (no Engerix B).

SCID-BM+Engerix: the chimeric mice were vaccinated with Engerix B (no transplantation of human PBL).

SCID-BM+Alum: the chimeric mice were vaccinated with Alum (no human PBL and no Engerix B).

Such fragments may be, for example, Fab or $F(ab)_2$ fragments obtained by digestion of the whole antibody with various enzymes as known and described extensively in the art. The antigenic characteristics of an antibody are determined by testing the binding of an antibody to a certain antigenic determinant using standard assays such as RIA, ELISA or FACS analysis.

Typically, the human monoclonal antibodies obtained by the method of the present invention have a relatively high affinity to HBVsAg being in the range of about 10^{-9}M to about 10^{-10}M as determined in a competative ELISA assay.

In accordance with a specific embodiment of the present invention there are provided hybridoma cell lines designated herein as *"18.5.1013"* and *"19.79.5"* which were deposited on May 22, 1996, in the European Collection of Cell Cultures (ECACC, CAMR, Salisbury, Wiltshire, SP40JG, U.K.) under Accession Nos. 96052170 and 96052168, respectively. Anti HBVsAg human monoclonal antibodies secreted by the above hybridoma cell lines and designated herein as *"Ab18.5.1013"* and *"Ab19.79.5"*, respectively, are also provided as well as fragments thereof retaining the antigen binding characteristics of the antibodies, and antibodies capable of binding to the antigenic epitope bound by *"Ab18.5.1013"* and *"Ab19.79.5"*.

The antigen bound by the antibodies defined above also constitutes an aspect of the invention.

Further aspects of the present invention are various diagnostic, prophylactic and therapeutic uses of the human anti HBVsAg monoclonal antibodies and the Ag bound by them. In accordance with this aspect of the invention, pharmaceutical compositions comprising the human anti HBVsAg monoclonal antibodies may be used for the treatment of chronic Hepatitis B patients by administering to such a patient a therapeutically effective amount of the monoclonal antibody or portion thereof capable of binding to the HBVsAg being an amount effective in alleviating the symptoms of the HBV infection or reducing the number of circulating viral particles in an individual. Such pharmaceutical compositions may also be used, for example, for immunization of new born babies against HBV infections or for immunization of liver transplantation patients to eliminate possible recurrent HBV infections in such patients. The antibodies of the invention may also be used in a method for the diagnosis of HBV infections in an individual by

mice from the Weizmann Institute Animal Breeding Center (Rehovot, Israel). All mice were fed sterile food and acid water containing cyprofloxacin (20 μg/ml) (Bayer, Leverkusen, Germany). Whenever necessary, mice were injected daily with 1 mg Fortum i.p. for five days post BMT (Glaxo Operations UK, Greenford, England).

Conditioning Regimens:

BALB/c mice were exposed to total body irradiation (TBI), from a gamma beam 150-A 60Co source (produced by the Atomic Energy of Canada, Kanata, Ontario) with F.S.D of 75 cm and a dose rate of 0.7 Gy/min, with 4 Gy followed 3 days later by 10-11 Gy (split dose).

Preparation and Transplantation of Bone Marrow Cells:

The femoral and tibial bones were removed from mice and homogenized in a sterilized 50 ml Omni-Mixer stainless steel chamber (Omni-Mixer Homogenize, Model No. 17106, OMNI International, Waterbury, CT. USA).
Recipient mice were injected i.v. with 4-6 x 10^6 of SCID/NOD bone marrow cells (in 0.2 ml PBS) immediately after irradiation.

Transplantation of Peripheral Blood Lymphocytes:

Peripheral blood lymphocytes (PBL) were obtained after informed consent by leukophoresis from donors positive for HBs antibodies and negative for HBV. PBLs were washed twice, counted and resuspended in PBS to the desired cell concentration.

100 x 10^6 human PBL were injected intraperitoneally (i.p.) into recipient mice, conditioned as described above. Control mice did not receive human PBL.

Immunization of the Chimeric Animals:

Mice were immunized once with hepatitis B vaccine (Engerix™-B; SB Biologicals Rixensart, Belgium) administered i.p. together with the PBL.

Cell and Plasma Collection from Human Mouse Chimera:

The black line represents the initial level of anti HBs antibodies in the serum of the human PBL donor.

Fig. 2 is a graphic representation showing the specific activity, i.e. the levels of anti HBVs antibodies per mg of human Ig in the sera of human donors (A–D, black columns) and the specific activity in the sera of chimeric mice transplanted respectively with human PBL of said donors (A–D, striped columns).

Fig. 3 is a graphic representation showing time response curve of anti HBs antibodies specific activity (mU/mg) in sera of chimeric mice (dotted line). The black columns represent the level of total human Ig (mg/ml), and the striped columns represent the level of specific anti HBs antibodies (mU/ml).

Fig. 4 is a graphic representation showing competitive inhibition of binding of anti HBs antibodies to HBs particles. The extent of binding was measured by ELISA using a horseradish peroxidase labeled anti human IgG secondary antibody. The anti HBs antibodies were diluted as indicated in the graph in medium (empty squares) or in 0.5 μg/ml HBs particles (black squares).

Fig. 5 is a photograph showing Hepatitis B infected liver sections stained with anti HBVs antibodies. All sections were stained with a *"secondary"* antibody, i.e. goat anti human Ig conjugated to biotin.

A – negative control. No first antibody.

B – positive control. First antibody – mouse anti HB antibody and a secondary anti–mouse Ig.

C – staining with anti HBs antibody No. 19.79.5.

D – staining with anti HBs antibody No. 18.5.1013.

Reference will now be made to the following Examples which are provided by way of illustration and are not intended to be limiting to the present invention.

EXAMPLES

MATERIALS AND METHODS

Mice:

Animals used were 6–10 weeks old. BALB/c mice were obtained from Harlan (Weizmann Institute Animal Breeding Center (Rehovot, Israel)), SCID/NOD

was stopped by addition of 10% sulfuric acid. Absorbance at 450 nm was quantified on an ELISA reader (Dynatech, Port Guernsey, Channel Islands, UK).

Concentration of antigen-specific human antibodies in mice sera was determined by HBsAb EIA kit (ZER, Jerusalem, Israel).

Human antibodies in hybridoma supernatants were determined by overnight incubation of supernatants on goat anti-human IgG+A+M (Zymed) coated plates, with goat anti-human IgG-peroxidase conjugated as the secondary reagent.

Antigen-specific antibodies in hybridoma supernatants were determined as above using Hbs antigen coated plates.

Determination of Human IgG Subclasses:

Human IgG subclasses were determined by sandwich ELISA using goat F(ab)2-purified anti-human IgG+IgM+IgA (Zymed Laboratories, San Francisco, CA) coated plates and Hbs antigen coated plates. Mouse anti-human IgG subclasses (Sigma) were used as second antibody and peroxidase-conjugated purified goat anti-human (Zymed Laboratories) as the detection reagent.

Statistic Analysis:

Statistical analysis was performed using the Stat View II program (Abacus Concepts, Inc., Berkeley, CA) on a Mackintosh Quadra 605 or Microsoft Excel 5.0 (Microsoft) on a 486 DX2 PC compatible. Student t-test, Anova correlation and regression analysis were utilized to calculate probability (p) and correlation coefficient (r) values. Results are presented as mean ± standard error.

Affinity Constant Measurements:

Determination of affinity constants (K_D) of the different anti-HBs antibodies to ad antigen (Chemicon Cat. No. AG 850) in solution were performed according to Friguet *et al.* (*Journal of Immunological Methods*, 77:305-319, 1985). The antigen at various concentrations ($3.5x10^{-10}$M to $1.4x10^{-9}$M) was first incubated in solution with a constant amount of antibody ($3.4x10^{-11}$M), in 0.1 M sodium phosphate buffer containing 2 mM EDTA and 10 mg/ml BSA, pH 7.8 (medium buffer). After o.n. incubation at 20 C the concentration of free antibody

Animals were bled from the retro-orbital vein using heparin-coated glass capillaries. Plasma was kept for human-Ig determination. Spleens were removed after the animals were sacrificed by cervical dislocation, cut into pieces and pressed through stainless steel sieves to make a cell suspension in PBS.

Cell Fusion:

Cells were mixed with the human-mouse heteromyeloma HMMA2.11TG/0 (Posner *et al. Hybridoma*, 6:611-625, 1987) at 3:1 ratio. Fusion was performed with 50% (w/v) PEG 1500 (Boehringer Manheim GmbH) in a standard procedure. Fused cells were seeded at a concentration of 30000 cells/well in 96-well U-bottom microtiter plates (Nunc, Denmark) in complete medium containing HAT-supplement (1x) (Biological Industries, Beit Haemek, Israel). Cells were fed with fresh HAT-medium a week latter. Two weeks after fusion supernatants were harvested for ELISA and medium was replaced with fresh HT-medium.

Hybridoma cultures secreting specific anti-HBs Ig were cloned at 0.5 cell/well in 96-well U-bottom microtiter plates.

Determination of Human Immunoglobulin:

Sera were tested for antigen specific and total human Ig. Total human Ig was quantified by sandwich ELISA using goat F(ab)2-purified anti-human IgG+IgM+IgA (Zymed Laboratories, San Francisco, CA) as the capture agent and peroxidase-conjugated purified goat anti-human (Zymed Laboratories) as the detection reagent. Human serum of known immunoglobulin concentration was used as the standard (Sigma, Rehovot,Israel). Microplates (Nunc, Roskilde, Denmark) pre-coated with the capture reagent (2.5 ug/ml, 50 ul/well) and blocked with 1% BSA were incubated overnight at 4C with dilutions of plasma from 1:20000 to 1:640000, or the standard from 0.2 to 0.06 ug/ml, then washed 5 times with PBS-Tween solution. The detection reagent was added and the plates were incubated for 1h at 37C, then washed again 3 times. Fresh substrate solution (TMB, Sigma) was added and, after peroxidase-catalyzed color development, the reaction

antibodies followed by biotinylated Goat anti-Human IgG (H + L) (Zymed, San Francisco, CA) using Histostain-SPTM kit (Zymed) according to the manufacture's recommendation. Control slides without using the 1st Human anti-HBs antibody were stained in parallel.

Example 1 Production of human anti HBs antibodies in chimeric mice

Human peripheral blood lymphocytes (PBL) from donors positive for anti HBs antibodies were implanted intraperitoneally into irradiated BALB/C mice which were radioprotected by transplantation of bone marrow from SCID mice. These chimeric mice were immunized with Hepatitis B vaccine (Engerix B) to induce a secondary immune response. The production of specific anti HBs antibodies along with total human Ig secretion was measured in mice sera. Fig. 1 shows levels of total human Ig and specific anti HBs antibodies in mice sera 14 days after transplantation of human PBL. Although the levels of human Ig secreted are similar in immunized and control mice, a strong specific immune response develops in mice vaccinated with hepatitis B vaccine as compared to the control group. Comparison of the levels of specific human antibodies produced in response to the antigen in immunized mice to their levels in the donors sera, indicates a 5-10 fold increase in the mice. Moreover, the specific activity measured in mice sera, i.e. the levels of anti HBs specific antibodies per mg of human Ig secreted, is 102-104 fold higher than the specific activity observed in the donor. This increase demonstrates a very high amplification of anti HBs antibody production in response to the antigen in the chimeric mice (Fig. 2). Production of human antibodies is detectable 10 days after immunization and reaches a plateau after three weeks. The specific activity is high at day 13 after immunization and decreases thereafter (due to increase in total human Ig secretion) (Fig. 3).

Example 2 Preparation and characterization of human monoclonal antibodies against HBs

Human B cells harvested from mice spleens two weeks after immunization were fused to human- mouse heteromyeloma cells (Posner *et al. Supra*). Hybridoma cells were tested for their growth rate, total Ig secretion and specific

was determined by an indirect ELISA. A volume of 300ul of each mixture were transferred and incubated for 2h at 20 C into the wells of a microtitration plate (Nunc) previously coated with Ad (50 μl/well at 1 μg/ml in 0.1 M $NaHCO_3$ buffer, pH 9.6 for 2 h at 37°C). After washing with PBS containing 0.04% Tween 20, the bound antibodies were detected by adding HRP-$F(ab')_2$ Goat anti human IgG (Zymed) diluted 1:3000 with medium buffer, 50 μl/well 2 h at 20°C. The plate was developed with TMB chromogen (Sigma T-3405 tablets) 50 μl/well. the reaction stopped with 10% H_2SO_4 50 μl/well and the plate read in an ELISA reader at 450 nm. The conditions were chosen so that the resulting f values (see Friguet *et al.*) were around 0.1. The antibody concentration used was deduced from an ELISA calibration done on the same plate. The affinity constant K_D was calculated from the relevant Scatchard plot.

Inhibition Assays:

The inhibition assay was performed in microtiter plates coated with HBs particles (2 μg/ml in PBS). The plate was blocked with 3% BSA in PBS. Hybridoma supernatants containing anti HBs antibodies were serially diluted. 50 μl of each dilution were added to the coated microtiter wells. Subsequently, 50 μl of HBs particles (ad/ay, 0.5 μl/ml in PBS) or PBS alone were added to each well. The plates were incubated overnight at room temperature in a humid chamber and washed 5 times with PBS-Tween. Next, 50 μl of goat anti human IgG conjugated to HRP (diluted 1:5000 in PBS) were added to each well. After a 4 hour incubation at room temperature in a humid chamber the plates were washed 5 times with PBS-Tween, and TMB was added to each well. Results were read using an ELISA reader, in a wavelength of 450 nm.

Immunohistostaining:

HBV positive liver fragment was fixed in 4% neutral buffered formaldehyde for 24 h and then embedded in paraffin using routine procedures. Sections of 4 μm thickness were cut from paraffin blocks and mounted on polylysine-coated slides. After deparaffinization and peroxidase quenching staining was performed using our monoclonal Human anti-HBs Protein A-purified

Table 2

Clone	Type	Production $\mu g/10^5$ cells/day	Kd (M)
18.5.1013	IgG1 Vλ	10.3	6.1×10^{-9}
19.79.5	IgG1 Vλ	5.8	1.62×10^{-9}

antibody production. Control fusion experiments were performed on the donor PBL that were activated in vitro with PWM and HBVsAg. Fusion frequencies in different experiments range from 0.9 -5 x 10-5. Most of the growing hybridoma clones secrete human Ig of which 0.1-4 % produce specific human anti HBs antibodies. Anti-HBs secreting hybridoma cells derived from chimeric mice spleens were compared to those obtained from fusion of the donors *in vitro* activated PBL in terms of Ig type and stability as seen in Table 1 below. The majority of the hybridomas from chimeric mice were found to be IgG type and all were stable for more than 12 months. In contrast, hybridomas derived from donor PBL were mostly unstable, only one clone has been stable for more than 12 months. Two stable hybridoma clones that secrete specific human anti HBs monoclonal antibodies were characterized. As seen in Table 2 below, these antibodies were purified on a protein A column as well as on an anti human Ig – agarose column and were both found to be of IgG1 subclass. Affinity constants range from $1.3x10^{-9}$ M to $6x10^{-9}$ M as tested by competitive ELISA. Specificity was tested by competitive inhibition assay using HB surface antigen of the ad-ay (1:1) subtype (Fig. 4). Fig. 5 shows specific binding of the human MoAbs of the invention to HBV by staining human liver fragments infected with HBV.

Table 1

Source of Hybridoma Cells	Anti-HBs Secretors		Stability
	IgG	IgM	
In Vitro Activated PBL	23 (48%)	25 (52%)	1 stable for > 10 months 17 unstable
Chimeric Mouse Splenocytes	6 (67%)	3 (33%)	6 stable for > 10 months 3 unstable

(a) the monoclonal antibody 18.5.1013 which is secreted by the hybridoma cell line deposited in the European Collection of Cell Cultures (ECACC) under Accession No. 96052170;

(b) an antibody capable of binding to the antigen which is bound by said 18.5.1013 antibody; and

(c) fragments of the antibodies of (a) or (b) which substantially retain the antigen binding characteristics of the whole antibodies.

8. A human monoclonal antibody being selected from the group consisting of:

(a) the monoclonal antibody 19.79.5 which is secreted by the hybridoma cell line deposited in the European Collection of Cell Cultures (ECACC) under Accession No. 96052168;

(b) an antibody capable of binding to the antigen which is bound by said 19.79.5 antibody; and

(c) fragments of the antibodies of (a) or (b) which substantially retain the antigen binding characteristics of the whole antibodies.

9. The hybridoma cell line deposited at the ECACC on May 22, 1996 under Accession No. 96052170.

10. The hybridoma cell line deposited at the ECACC on May 22, 1996 under Accession No. 96052168.

11. An antigen bound by an antibody according to Claim 7 or 8.

12. A pharmaceutical composition for the treatment of HBV infections comprising as an active ingredient an antibody in accordance with Claims 5, 7 or 8 together with a pharmaceutically acceptable carrier.

13. A method for the treatment of HBV infections comprising administering to an individual in need a therapeutically effective amount of antibodies according to Claims 5, 7 and 8.

14. A method for the prevention of HBV infections comprising administering to an individual antibodies in accordance with Claims 5, 7 or 8 to prevent further infection of the treated individual with HBV.

15. A method for the prevention of HBV infections comprising immunizing an individual with an antigen according to Claim 11.

CLAIMS:

1. A process for obtaining human monoclonal antibodies (hMoAb) capable of binding to Hepatitis B virus surface antigen (HBVsAg) comprising:

(a) immunizing a chimeric rodent M4 having xenogeneic hematopoietic cells with Hepatitis B surface antigen HBVsAg such that xenogeneic antibody-producing cells are produced in said rodent, wherein said rodent M4 is a rodent M1, the hematopoietic cells of which have been substantially destroyed, said rodent M1 having transplanted therein hematopoietic cells derived from a mouse M2 having a hematopoietic deficiency, and xenogeneic hematopoietic cells derived from human M3;

(b) removing and immortalizing said antibody-producing cells;

(c) selecting and cloning the immortalized antibody producing cells producing the antibodies capable of binding to HBVsAg and;

(d) isolating the antibodies produced by the selected, cloned immortalized antibody producing cells.

2. A process according to Claim 1, wherein the rodent M1 is a BALB/C mouse and the mouse M2 is a SCID mouse.

3. A process according to Claim 1 or 2, wherein the human M3 is human having a high level of anti HBVsAg antibody and said xenogeneic hematopoietic cells derived from human M3 are peripheral blood lymphocytes (PBL).

4. A process according to Claims 1–3, wherein the Hepatitis B surface antigen is Engerix™-B vaccine.

5. A human monoclonal antibody obtained by the process of Claim 1 and fragments thereof substantially maintaining the antigen binding characteristics of said antibodies.

6. A hybridoma cell line producing a human monoclonal antibody in accordance with Claim 5.

7. A human monoclonal antibody being selected from the group consisting of:

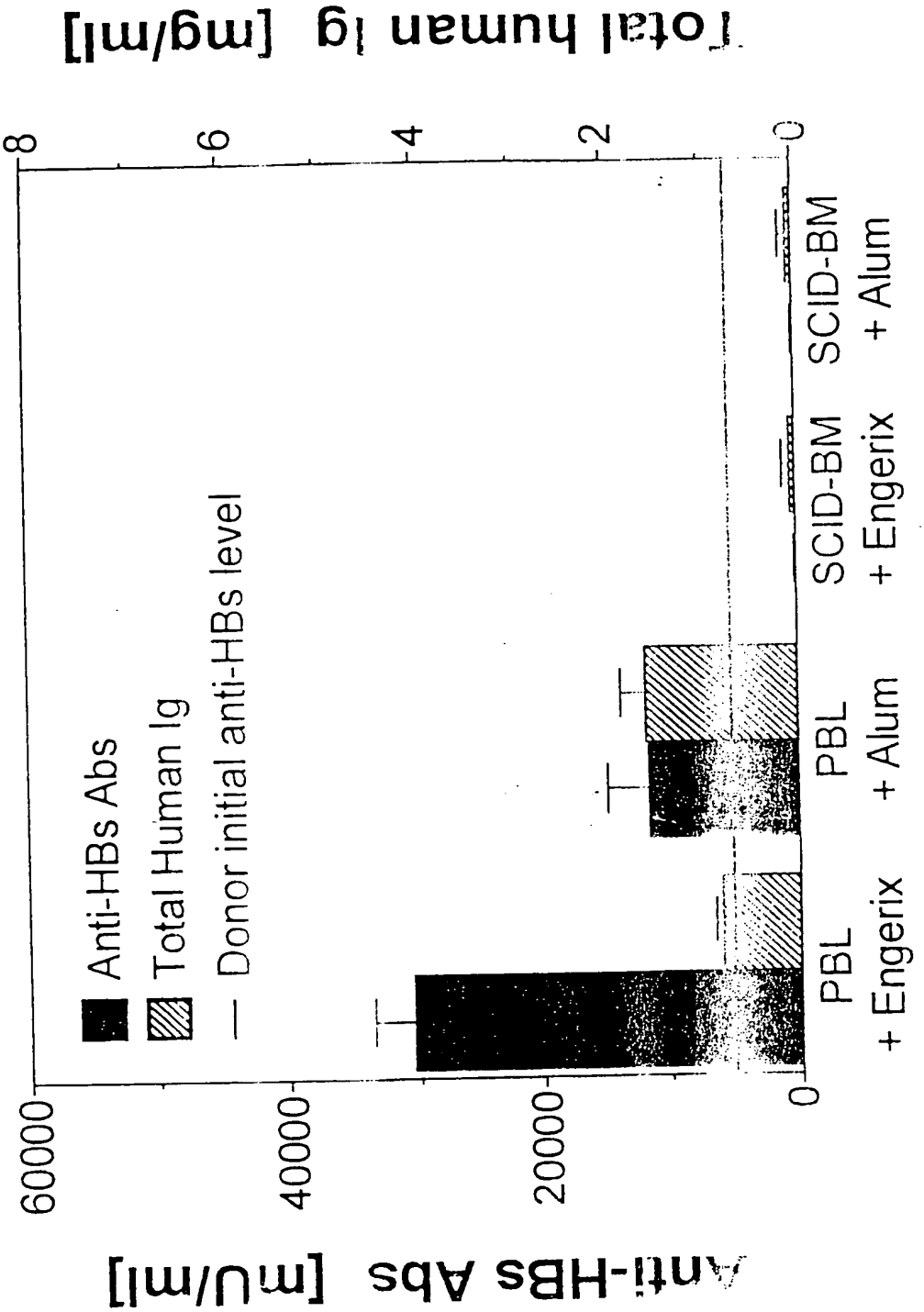

Anti-HBs Abs [mU/ml]
60000
40000
20000
0
Total human Ig [mg/ml]
8
6
4
2
0
Anti-HBs Abs
Total Human Ig
Donor initial anti-HBs level
PBL + Engerix
PBL + Alum
SCID-BM + Engerix
SCID-BM + Alum

Figure 1

16. A method for the diagnosis of HBV infections in a body fluid sample comprising:

(a) contacting said sample with an antibody of any of Claims 5, 7 or 8 under conditions enabling the formation of antibody-antigen complexes;

(b) determining the level of antibody-antigen complexes formed;

a level significantly higher than that formed in a control sample indicating an HBV infection in the tested body fluid sample.

17. A method for detecting anti HSV antibodies in a body fluid sample comprising:

(a) contacting the sample with the antigen of Claim 11 under conditions enabling the formation of antibody-antigen complexes;

(b) determining the level of antibody-antigen complexes formed; and

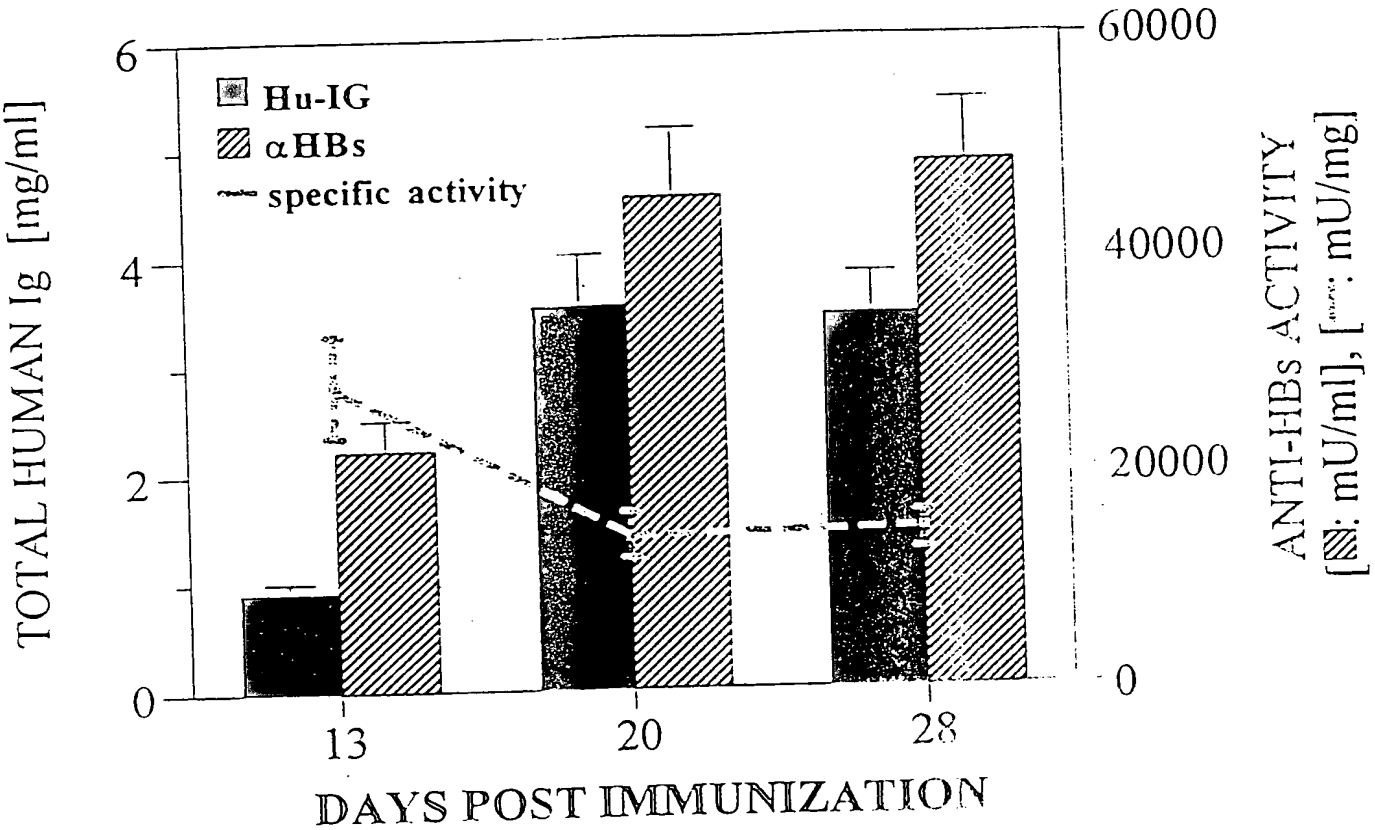
(c) indicating the presence of HSV antigens in the tested body fluid sample.

18. A kit for use in the method of Claim 16.

19. A kit for use in the method of Claim 17.

For the Applicants,
REINHOLD COHN AND PARTNERS
By: A. B. Cohn

Figure 3


TOTAL HUMAN Ig [mg/ml]
ANTI-HBs ACTIVITY [▨: mU/ml], [---: mU/mg]
Hu-IG
αHBs
specific activity
0
2
4
6
0
20000
40000
60000
13
20
28
DAYS POST IMMUNIZATION

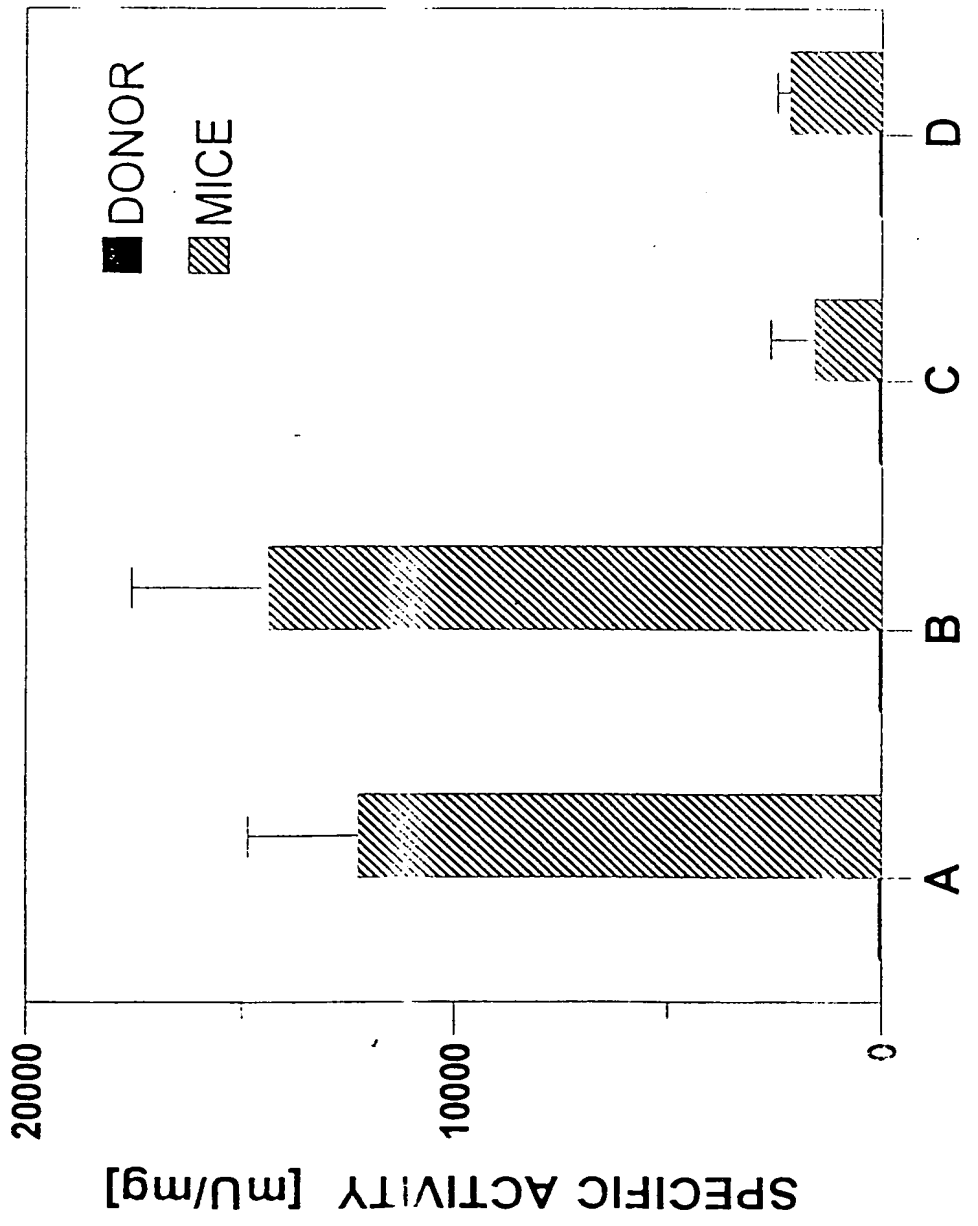


Figure 2

Figure 2

FIGURE 5

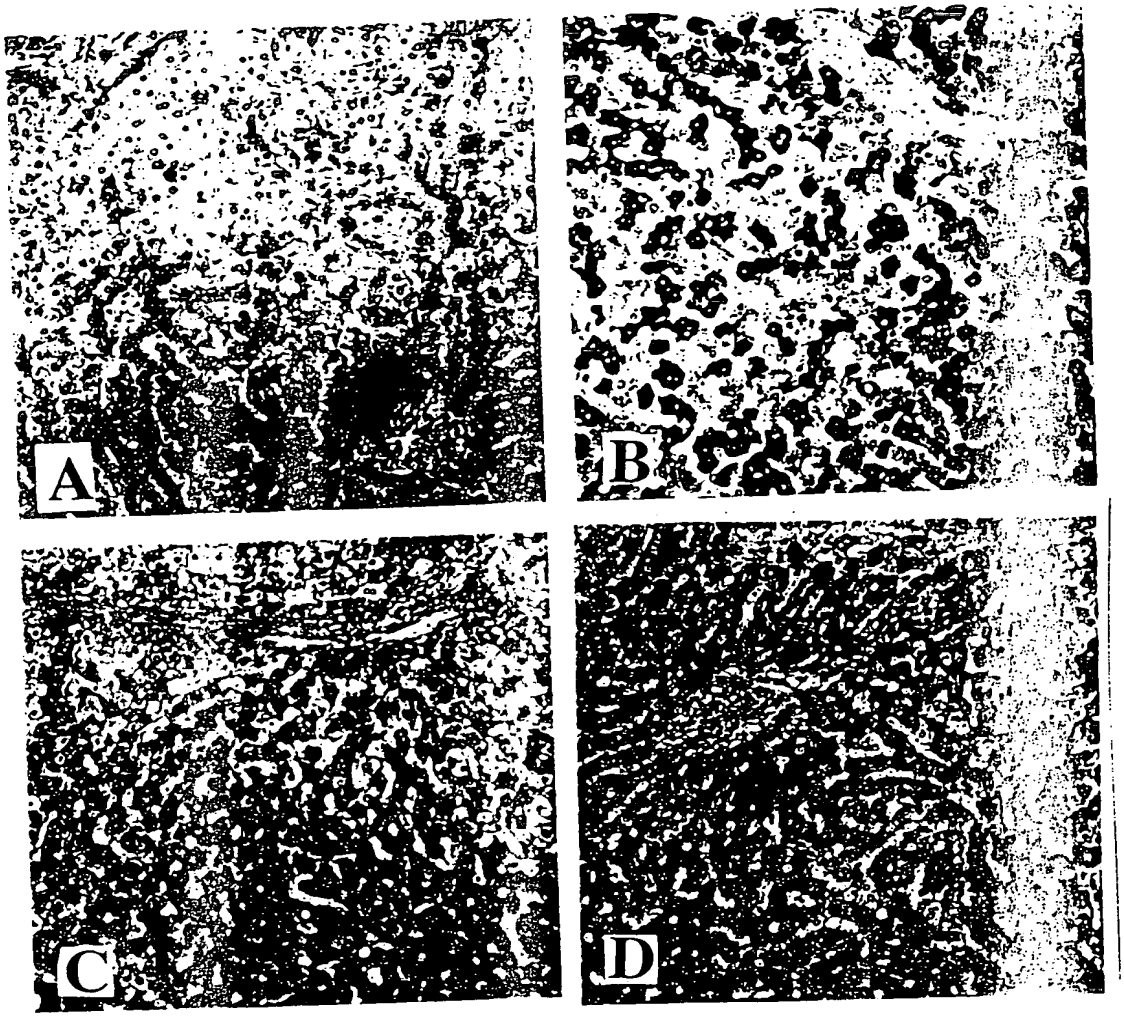
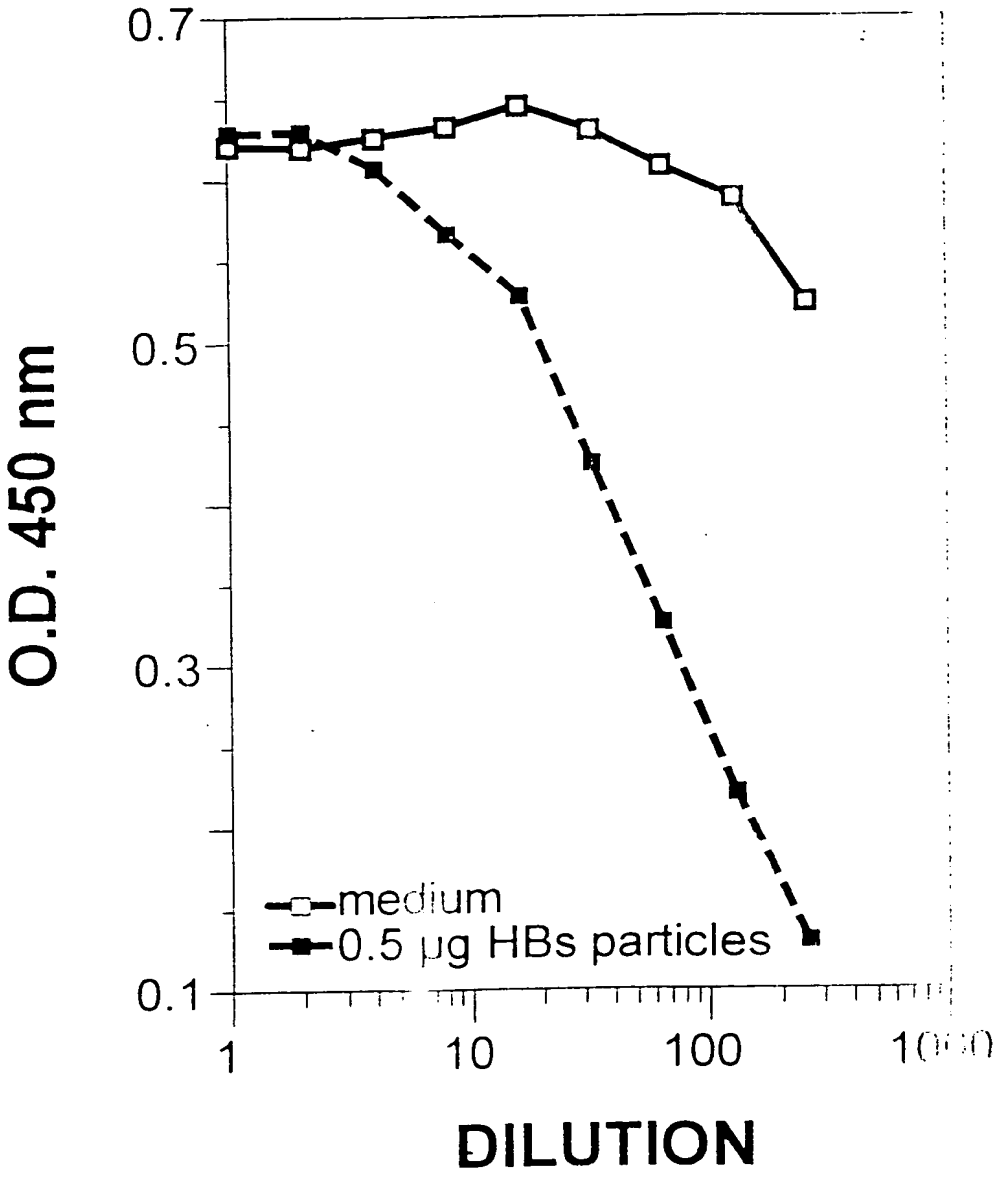


Figure 4


0.7
0.5
0.3
0.1
O.D. 450 nm
medium
0.5 µg HBs particles
1
10
100
DILUTION